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RESEARCH ARTICLE

NEO VASCULARISATION IN CHRONIC WOUNDS WITH AMNIOTIC MEMBRANE DRESSING

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ABSTRACT

Background: Amniotic membrane from placenta has unique properties likeenhanced wound healing through neovascularisation, analgesic, antiadhesive effects, bacteriostatic, without inducing immunological reactions.
Aim: to quantify the neo vascularisation that occurs by applying amnioticmembrane as dressing in chronic non healing ulcers
Material and Method: This is a randomized prospective pilot study done to evaluate effects of healing in chronic ulcers as evidenced by quantum of neo vascularisation by amniotic membrane application for a period of eight weeks in 15 patients. The amniotic membrane harvested from caesareansection is applied over ulcers and the patients are evaluated at first, second, fourth, sixth and eighth week.
Results: There is 61% of reduction in ulcer size (p-value 0.000) with twocompletely healed ulcers at the end of study period. In the first two weeks there is faster rate of neovascularisation as seen by counting the numberof capillaries in an average of five high power fields (p-value 0.000), lateron there is congestion of vessels with formation of granulation tissue whichpeaks at fourth week (p-value 0.000), leading to reduction in ulcer size. Significant analgesia is found in all the patients (p-value 0.000).
Conclusion: Amniotic membrane application enhanced neo vascularization in eight weeks time there by early healing of wound. The costeffectiveness, readily available, ease of application and better scarformation makes it a better choice for treating chronic ulcers.

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INTRODUCTION

The chronic non healing wounds are highly prevalent and have major impact on quality of life of people increasing the morbidity apart from economic consequences due to its refractory nature. There are various methods of wound care like conventional dressing with saline, foams and sprays, films, vacuum assisted dressing, biological dressing with amniotic membrane, collagen etc.Amniotic membrane, the innermost layer of placenta can be used to treat non healing ulcers as it possesses many properties which are ideal for wound coverage like enhancement of wound healing by neovasularisation later on added by re-epitheliasation, decrease the bacterial count in the wound, good adherence to the wound thereby having analgesic effects, decrease the fluid loss from the wound.It is found to be immunologically inert as it does not express HLA-A, B, C and DR or β2 micro globulin, hence graft rejections are negligible. (Chen and Tofe, 2010) Based on these properties and other advantages like easy availability, cost effectiveness and healing with better scar formation; a pilot study was conducted on 15 patients with chronic non healing ulcers.

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MATERIALS AND METHODS

This is a randomized prospective pilot study done on fifteen patients with chronic non healing ulcers of any non malignantetiology like diabetes, varicose vein, traumatic etc involving either gender with age between 30yrs to 60yrs.The size of ulcer being 4*4cms or more (i.e. equal to or more than 16cm^2 surface area of the ulcer) with no tendency of healing in past two months despite conventional treatmentand with no other co-morbid conditions like deep vein thrombosis, arterial insufficiency, neuropathy, severe systemic diseases etc. Informed consent is taken from the patients. Debridement of ulcer is done to remove necrotic slough. The initial ulcer size is measured at its maximum diameters by using 0.5*0.5cm graph printed over transparent sheath up to one decimal in centimetres.Initial biopsy of 0.5cm is taken from floor of ulcer and sent for histopathology in 2% formalin solution. Routine histopathology of the specimen is carried out and under 40x

magnification, the number of blood vessels present per field is counted at four corners of the field and centre; and average of five fields is taken. Amniotic membrane is obtained from patients undergoing elective Caesarean section whoare not positive for HIV and HBsAg and other STD. The amniotic fluid should be clear with no meconium staining.Extraction of amniotic membrane: After delivering the baby from uterus, placenta with its covering is taken into a sterile tray. The membranous coverings are cut with scissors. The amniotic membrane is separated from chorion and placed in another sterile tray. The separated amniotic membrane is thoroughly washed with normal saline and such freshly harvested membranes are used for clinical application.

Evaluation of wound

Wound is inspected at 1st, 2nd, 4th, 6th, and 8th weeks. Healing is measured by taking digital photo with Sony coolpix camera using 4x magnification from a distance of 20cms. Biopsy is taken from the wound and subjected to histopathogy examination as done initially during the above mentioned weeks. The number of new blood vessels formed are counted in five fields and average is taken. The vascular congestion and stroma formation are also noted.The pain pattern of the ulcer after application of amniotic membrane are demonstrated using visual analogue scale.

Statistical analysis

The results are analysed by chi-square test and p-value calculated using SPSS software.The p- value of <0.05 is taken as significant for the purpose of this study.

RESULTS

The role of amniotic membrane on chronic non healing ulcers is evaluated for a period of eigt weeks and the parameters taken into consideration include changes in ulcer size, changes in the histopathological examination and analgesic effects.

1. Changes in ulcer size: (Table 1)

The mean ulcer size at the beginning is 37.6cm^2 which gradually decreased and by the end of study period of 8 weeks it was 14.6cm^2 i.e. significant reduction in ulcer size of 61% with two complete healing of ulcers (p-value 0.000).

2. Changes in histopathological examination: (Table 2)

a) Neovascularisation :

There were very few capillaries in every high power field (hpf) at the beginning ofstudy. After amniotic membrane dressing a hike is seen in number of capillaries in first two weeks of study (Figure-1). This rise continued in a slower pace for the next two weeks and from then at around sixth week onwards the rate of increase in capillaries remained almost static (p-value 0.000).

b) Congestion of blood vessels: (Table 3)

Apart from increase in number of capillaries, congestion around these newly formed blood vessels is seen from the end of second week.

c) Formation of granulation tissue:

From the end of second week, along with congestion of blood vessels, granulation tissue started to form which further increased gradually. The rate of formation of stroma was so rapid from the end of fourth week onwards (Figure 2) which resulted in high amount of stroma by the end of the study (p-value 0.000) (Figure 3).

3) Analgesic effects: The severity of the pain started to decrease from the end of second week onwards and by the end of the study, patients experience very mild pain (p-value-0.000).

Table 1. Change in Ulcer size after Amniotic membrane application

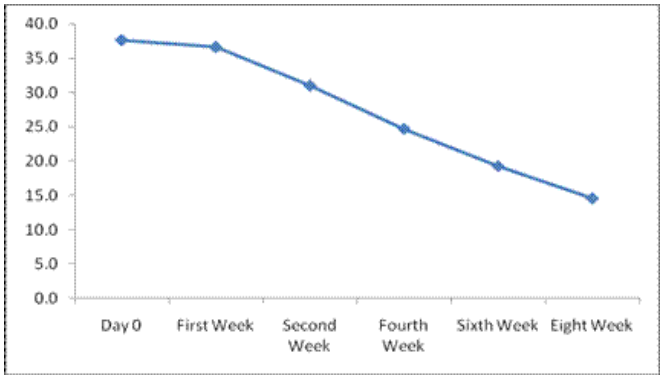


Table 2. Growth of new capillaries

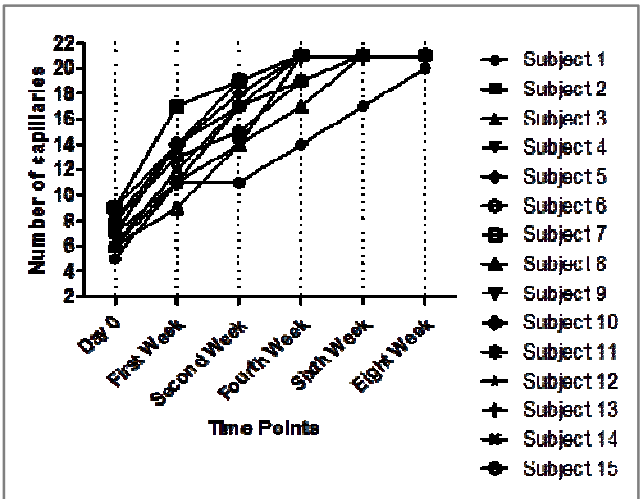
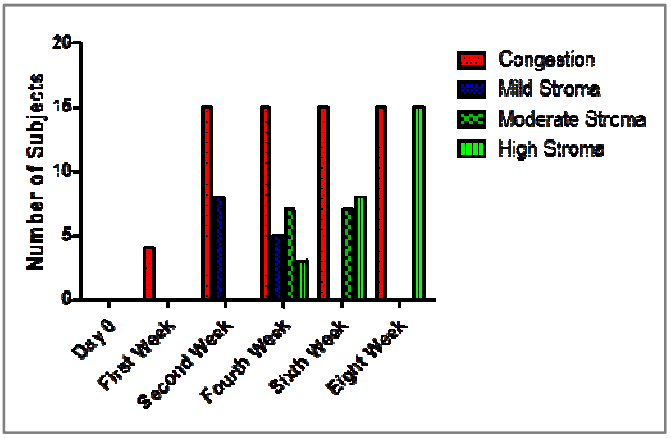


Table 3. Congestion of blood vessels and stroma formation



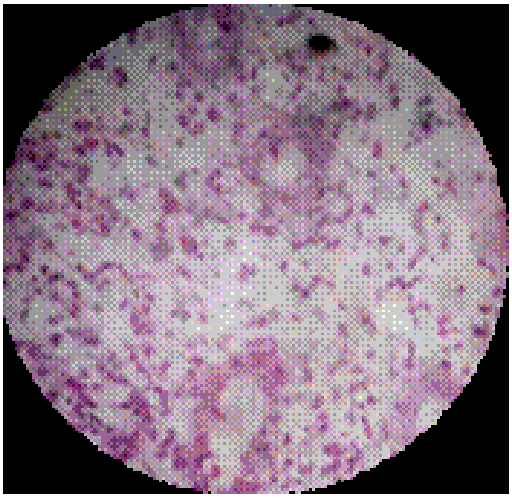


Fig.1. 1st week 10-12 capillaries/hpf

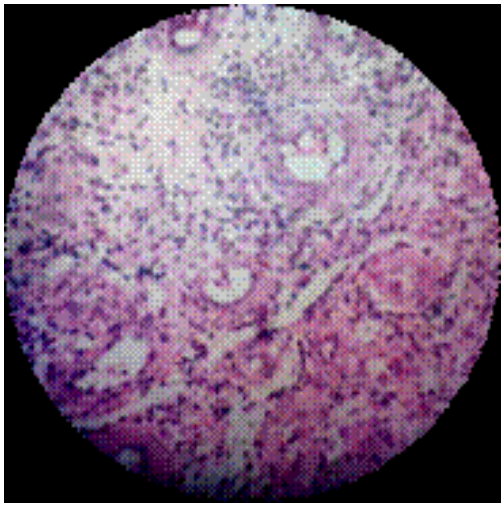


Fig.2. 4th week 15-18 capillaries/hpf with beginning of stroma formation

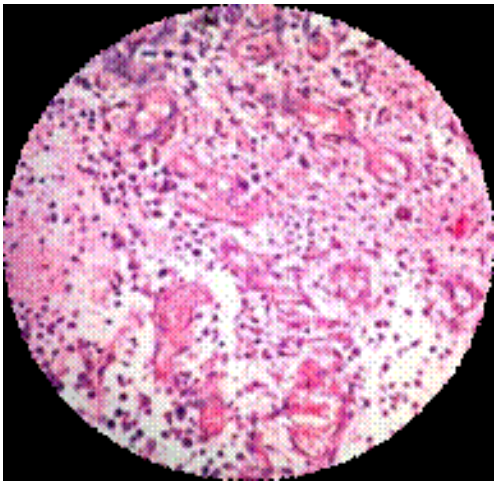


Fig.3. 8th week background Congested >20 capillaries/hpf, with high stroma

DISCUSSION

The amniotic membrane is in use for the treatment of wounds since 100yrs. Davis, in 1910, reported a comprehensive review of 550 cases at Johns Hopkins University (Davis, 1910). Sabella and Stern, in 1913, have reported use of preserved amniotic membrane over burns and ulcers. (Sabella, 1913; Stern, 1913) In 1940, Deroth was the first to employ amniotic membrane to repair eye wounds. (De Roth, 1940) Since then till date, there has been ample use of amniotic membrane transplantation for different types of wounds like traumatic, diabetic, venous ulcers, burn wounds etc. It is because of some unique characteristic properties which helps in speeding up the healing process and thereby decreasing the wound size.

They are:

Ultrastructural characters: The amniotic epithelium contains intracellular cytoskeletal filaments like actinin, desmoplakin, cytokeratin, vimentinetc which has role in structural integrity and modulation of shape of cell of healing tissue. (Bani-Yaghoub *et al*., 2012) The stromal portion contains many proteoglycans and glycoproteins like laminin which has a significant role in maintenance and promotion of tissue phenotype, invasion of fibroblasts and angiogenesis in wound healing.

Immunomodulative and immune privilege: Amniotic membrane is found to be immunologically inert as it do not express HLA-A, B, C and DR or β2 micro globulin, hence graft rejections are negligible. (Akle *et al*., 1981)

Antimicrobial effect: The amniotic membrane forms a tight adherence with the wound surface due to elastic and fibrin linkages thus help in restoring lymphatic integrity and allow faster removal of surface debris and bacteria from the wound. It secretes antimicrobial and immunomodulative factors which upregulates bacterial killing by immune cells. Defensins, a bactericidal product and cystatin E, an antiviral agent are released in large amounts by the membrane. (King *et al*., 2007)

Pain reduction: Amniotic membrane has the ability to reduce pain by its anti inflammatory property, good hydration to the wound bed to promote faster healing and protecting the exposed nerve endings to external stimulus due to its tight adherence property. (Chopra and Thomas, 2013)

Antiscarring and anti inflammatory effect: During the process of wound healing, fibroblast are activated by TGF-beta secreted by macrophages. Amniotic membrane downregulates TGF-beta and maintain proper balance between TGF-1 and TGF-3, that prevents fibrosis and scarring. Amniotic membrane decreases the secretion of pro inflammatory cytokines like TNF-alpha, interferon etc and increase anti inflammatory cytokines IL-10, 4 etc. Various immune cells like T cells, dendritic cells, B cells are actively suppressed. (Chopra and Thomas, 2013)

Angiogenesis: There is induction of vascular endothelial growth factor (VEGF) from the cells of amniotic membrane which results in formation of new blood vessels, thereby increasing granulation tissue and promotes wound healing. (Burgos, 1983)

Increase in extracellular matrix deposition:

Amniotic membrane fastens the process of wound healing by accelerating the change from inflammatory to proliferative phase. Hence there is increase in rate of granulation tissue formation and epithelialisation due to early start up of the process leading to increased extracellular matrix deposition.Gruss JS and Jirch DW in the year 1978 have

studied the versatile properties of biological dressing with amniotic membrane over 120 patients. (Gruss and Jirch, 1978) Isabelle Mermet, Nathalie Pottier (2007) from university de Franche-Comte, France have studied the use of amniotic membrane transplantation in venous ulcers and found that 80% of patients have significant decrease in size of ulcer (more than 50% reduction) in a period of 3months. (Isabelle Mermetand Nathalia Pottier, 2007) A study done by Hanumanthappa MB, Gopinathan S (2012) from AJ Institute of Medical Sciences, Mangalore shows that there is faster rate of reduction of wound size from 3rd week after application of amniotic membrane when compared with normal saline dressing of the wound. (Hanumantappa *et al*., 2012) Zelen, Serena *et al*. (2013) have done a prospective randomized comparative parallel study of amniotic membrane wound graft in the management of diabetic foot ulcers and have noticed a superior healing property of wound. (Zelen *et al*., 2013) Vivekananda *et al* (2014) have done a research on effectiveness of amniotic membrane dressing and concluded that it was superior to conventional dressing in terms of epithelialization, graft uptake and prevention of infection at ulcer sites. (Vivekananda *et al*., 2014)

Conclusion

The amniotic membrane application enhanced wound healing by increasing the number of capillaries in the wound. Rapid neovascularisation is seen in first two weeks of application, followed by slow increase in number of capillaries with congestion around newly formed blood vessels.This increase in blood vessels enhances formation of stroma and granulation tissue there by leading to reduction of wound size.
The severity of pain is reduced within one to two weeks of application of amniotic membrane over wound.Other advantages of this membrane include immunologically inert, cost effectiveness, readily available, ease of application and better scar formation.

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